

# 5<sup>th</sup> World Congress on **Cell & Stem Cell Research**

March 23-25, 2015 DoubleTree by Hilton Chicago - North Shore, USA

## **TNF modulation of cancer stem cells in renal clear cell carcinoma**

Rafia SAI-Lamki, Jun Wang, Simon Pacey, Peter Vandenabeele, Jordan Pober and John R Bradley  
Cambridge Biomedical Campus, United Kingdom

**T**umor necrosis factor alpha (TNF), signaling through TNFR2, may act as an autocrine growth factor for renal tubular epithelial cells. Clear cell renal carcinomas (ccRCC) contain cancer stem cells (CSCs) that give rise to progeny which form the bulk of the tumor. CSCs are rarely in cell cycle and, as non-proliferating cells, resist most chemotherapeutic agents. Thus recurrence after chemotherapy may result from the survival of CSCs. Therapeutic targeting of both CSCs and the more differentiated bulk tumor populations may provide a more effective strategy for treatment of RCC. In this study, we hypothesized that TNFR2 signaling will induce CSCs in ccRCC to enter cell cycle so that treatment with ligands that engage TNFR2 will render CSCs susceptible to chemotherapy. To test this hypothesis, we have utilized wild-type TNF (wtTNF) or specific muteins selective for TNFR1 (R1TNF) or TNFR2 (R2TNF) to treat either short term organ cultures of ccRCC and adjacent normal kidney (NK) tissue or cultures of CD133+ cells isolated from ccRCC and adjacent NK, hereafter referred to as stem cell-like cells (SCLCs). The effect of cyclophosphamide (CP), currently one of the most effective anticancer agents, was tested on CD133+SCLCs from ccRCC and NK before and after R2TNF treatment. Responses to TNF were assessed by flow cytometry (FACS), immunofluorescence, and quantitative real time PCR, TUNEL and cell viability assays. Cytotoxic effect of CP was analysed by FACS using Annexin V-FITC and propidium iodide. In addition, we assessed the effect of TNF on isolated SCLCs differentiation using a three-dimensional (3D) culture system. Clinical samples of ccRCC contain a greater number SCLCs compared to NK and the number of SCSC increases with higher tumor grade. Isolated SCLCs show expression of stemness markers (oct4, Nanog, Sox2, Lin28) but not differentiation markers (cytokeratin, CD31, CD45 and EpCAM). In ccRCC organ cultures, wtTNF and R2TNF increase CD133 and TNFR2 expression and promote cell cycle entry whereas wtTNF and R1TNF increase TNFR1 expression and promote cell death of SCLCs. Similar findings are observed in SCLCs isolated from NK but the effect was greater in SCLCs isolated from ccRCC. Application of CP distinctly triggered apoptotic and necrotic cell death in SCLCs pre-treatment with R2TNF as compared to CP treatment alone, with SCLCs from ccRCC more sensitive to CP compared to SCLCs from NK. Furthermore, TNF promotes differentiation of SCLCs to an epithelial phenotype in 3D cultures, confirmed by cytokeratin expression and loss of stemness markers Nanog and Sox2. The differentiated cells show positive expression of TNF and TNFR2. These findings provide evidence that selective engagement of TNFR2 drive CSCs to cell proliferation/differentiation, and targeting of cycling cells with TNFR2 agonist in combination with anti-cancer agents, may be a potential therapy for ccRCC.

### **Biography**

Rafia Al-Lamki is a Clinical Scientist in the Department of Medicine, University of Cambridge. She has been educated in Kenya, Oman, USA and the UK. Rafia graduated with a BSc (Hons) in Applied Biological/Biomedical Sciences at the University of the West of England in Bristol in 1994 before gaining an MPhil/Ph.D. in Cellular Pathology at the University of Cambridge, Fitzwilliam College in 1999. She has received several awards; the Oman-American Joint Commission Scholarship in Advanced Medical Laboratory Sciences, University of California, San Francisco, USA, Oman Ministry of Health-British Council scholarship, Leatherseller's Prestigious Award for highest achiever during her BSc studies, training fellowship in Cellular Pathology from the World Health Organisation and the Cambridge Commonwealth / Overseas Trust bursary. She was appointed a Research Associate in the Department of Medicine in 2000, joined St Edmund's college as a Bye-Fellow in 2004, and became a Senior Research Associate in 2005 and Clinical Scientist in 2007. Rafia's research focuses on the role of the inflammatory mediator tumour necrosis factor, in renal disorders and cancer, and cardiovascular disease. She has developed a unique tissue organ culture model, which has provided novel insights into molecular and cellular responses in kidney and heart tissue, findings of which have contributed to various publications and a patent invention. She has also recently established a novel model system for isolation and culture of cancer stem cells from human kidney tissue. She is an active participant in the Cambridge-Yale Cardiovascular Research Programme; Editorial board member of various journals, recently co-authored a book 'The TNF Superfamily' and a fellow of several prestigious organisations. Solely, Rafia self-raised £70,000 to fund her MPhil/Ph.D. studies in the University of Cambridge. She is fluent in English, Kiswahili and understands Arabic. Her interests include travelling, reading, running, windsurfing, squash, tennis, netball and dancing. She has assisted in coaching and organising the Omani Nurses team in the Muscat Ladies Netball league of Oman.

[rsma2@hermes.cam.ac.uk](mailto:rsma2@hermes.cam.ac.uk)